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Revisiting modes of energy generation in sulfate reducing bacteria

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Ecosystems and Networks Integrated with Genes and Molecular Assemblies

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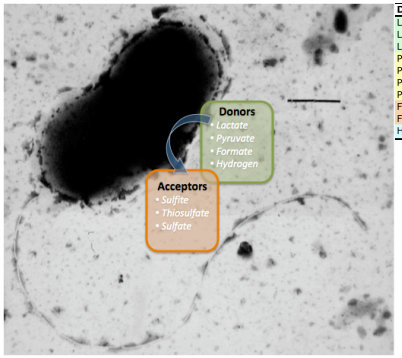
Abstract

Sulfate reducing bacteria (SRB) play an important role in global sulfur and carbon cycling through their ability to completely mineralize organic matter while respiring sulfate to hydrogen sulfide. They are ubiquitous in anaerobic environments and have the ability to reduce toxic metals like Cr(VI) and U(VI). While SRB have been studied for over three decades, bioenergetic modes of this group of microbes are poorly understood. *Desulfovibrio vulgaris* strain Hildenborough (DvH) has served as a model SRB over the last decade with the accumulation of transcriptomic, proteomic and metabolic data under a wide variety of stressors. To further investigate the three hypothesized modes of energy generation in this anaerobe we conducted a systematic study involving multiple electron donor and acceptor combinations for growth. DvH was grown at 37°C in a defined medium with (a) lactate + thiosulfate, (b) lactate + sulfite (c) lactate + sulfate, (d) pyruvate + sulfate, (e) H2 + acetate + sulfate, (f) formate + acetate + sulfate, g) formate + sulfate and (h) pyruvate fermentation. Cells were harvested at mid-log phase of growth for all conditions for transcriptomics, when the optical density at 600nm was in the range 0.42-0.5. Initial results indicate that cells grown on lactate do not appear to significantly differentiate their gene expression profiles when presented with different electron acceptors. These profiles however differ significantly from those observed during growth with other electron donors such as H2 and formate, as well as during fermentative growth. Together the gene expression changes in the presence of different electron donors provide insights into the ability of DvH to differentially reduce metals such as Cr(VI). Here we present revised modes of energy generation in DvH in light of this new transcriptomic evidence.

Design



Donor	Acceptor
Lactate	Sulfate
Lactate	Thiosulfate
Lactate	Sulfite
Pyruvate	Sulfate
Pyruvate	Thiosulfate
Pyruvate	Sulfite
Pyruvate	(Fermentation)
Formate	Sulfate
Formate + Acetate	Sulfate
Hydrogen + CO_2	Sulfate

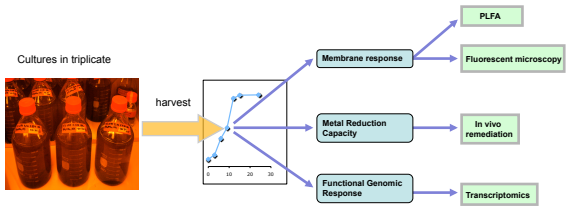
Cells of DvH were grown under the above set of electron donors and acceptors

Schematic representation of the experimental plan:

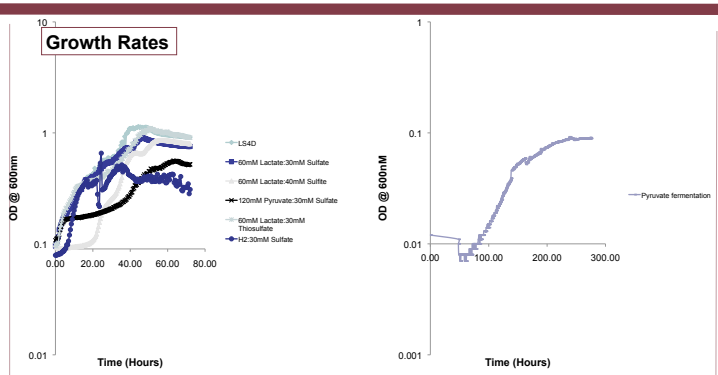
3 cultures grown to mid-log phase for each media variation

Samples harvested for 3 categories of investigation:

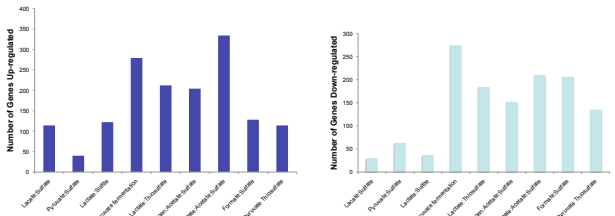
- Membrane response, metal reduction and genomic response

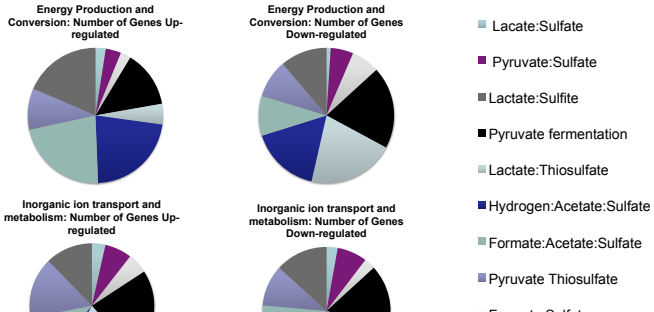


Growth Rates



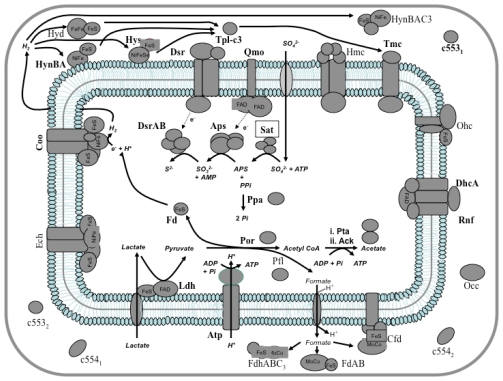
Transcriptomic Responses





Under each condition, gene expression was compared to culture grown in the defined medium LS4D. We show here the gene function categories with interesting differences between the different conditions. To determine if the change in gene expression was statistically significant, a Z value of 1.5 was used as a cut-off. The Z value was calculated using the following equation:

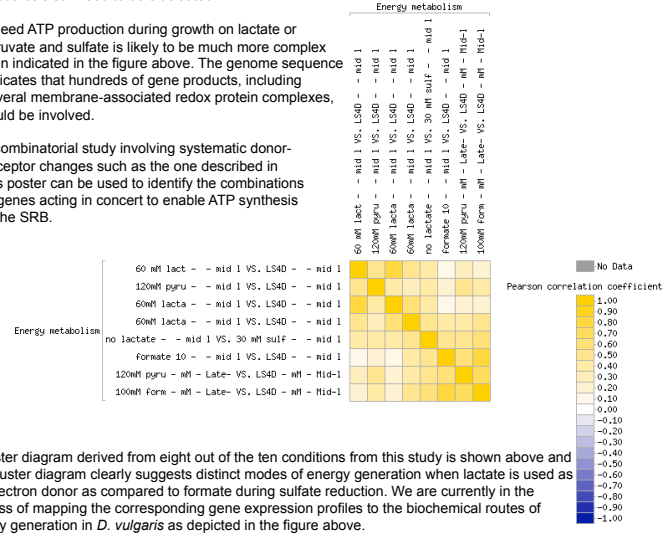
$Z = (\log_2(\text{treatment/control}))/(0.25+\sum\text{variance})^{1/2}$



Desulfovibrio spp. derive energy for growth from redox reactions in which electron donors such as organic acids, alcohols, or hydrogen are oxidized by electron acceptors such as sulfate, thiosulfate, or sulfite. ATP synthesis may proceed by multiple mechanisms as shown in the figure above for growth on lactate, pyruvate, formate, or hydrogen and sulfate. ATP formed by substrate level phosphorylation equals that required for activation of sulfate. Hence growth on lactate- and sulfate-containing medium would be expected to require proton motive force-driven ATP synthesis. The hydrogen cycling hypothesis proposed by Odom and Peck suggested that protons and electrons generated during lactate and pyruvate oxidation are converted to hydrogen by a cytoplasmic hydrogenase and that this H_2 diffuses to the periplasm, where it is reoxidized. However, the pathway components involved in this cycling hypothesis still need to be elucidated.

Indeed ATP production during growth on lactate or pyruvate and sulfate is likely to be much more complex than indicated in the figure above. The genome sequence indicates that hundreds of gene products, including several membrane-associated redox protein complexes, could be involved.

A combinatorial study involving systematic donor-acceptor changes such as the one described in this poster can be used to identify the combinations of genes acting in concert to enable ATP synthesis in the SRB.



A cluster diagram derived from eight out of the ten conditions from this study is shown above and this cluster diagram clearly suggests distinct modes of energy generation when lactate is used as the electron donor as compared to formate during sulfate reduction. We are currently in the process of mapping the corresponding gene expression profiles to the biochemical routes of energy generation in *D. vulgaris* as depicted in the figure above.

Reference

Odom, J. M., and H. D. Peck, Jr. 1981. "Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria Desulfovibrio sp." FEMS Microbiol. Lett. 12:47–50.

ACKNOWLEDGEMENTS

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